Transplantation of Suboptimal Donor Livers: Exploring the Boundaries

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10 Summary, Discussion and Future Perspectives

SUMMARY PART 1

The first part of this thesis contains a review and three observational studies on identifying the boundaries of transplantation of suboptimal donor livers.

After a general introduction in **chapter 1**, a review was presented in **chapter 2** focusing on biliary complications after liver transplantation. Bile leaks and bile duct strictures (anastomotic or non-anastomotic) are the most common types of biliary complication. Of these, non-anastomotic strictures (NAS) are the most troublesome and difficult to treat. Patients receiving a donation after circulatory death (DCD) liver graft, living donor graft, or a split liver graft are at increased risk of developing biliary complications. Although ischemia and subsequent reperfusion injury of the bile ducts is a main risk factor for the development of NAS, other mechanisms, such as immune-mediated injury, bile salt toxicity, and insufficient regeneration of the epithelium also play a role in the pathogenesis.

In **chapter 3** the results of a nationwide retrospective study on the association between donor hepatectomy time and the development of biliary injury during DCD liver transplantation are presented. Donor hepatectomy time was defined as the period from the initiation of cold flushing until the liver was removed from the abdominal cavity and placed into a bowl with ice-cold preservation solution. The impact of donor hepatectomy time on biliary injury was studied in a chronological fashion. First, it was observed in human bile duct biopsies that donor hepatectomy time negatively influenced the degree of bile duct injury prior to transplantation. Secondly, livers with prolonged hepatectomy time produced bile of inferior quality during normothermic machine perfusion, displayed by lower biliary bicarbonate secretion and therefore a lower bile pH. Lastly, in a nationwide retrospective database study, donor hepatectomy time was identified as an independent significant risk factor for the development of NAS after DCD liver transplantation. In conclusion: donor hepatectomy time influences the development of biliary injury in DCD liver transplantation. The results from this study suggest that donor hepatectomy time should be kept as short as possible to reduce rates of post-transplant cholangiopathy.

Chapter 4 contains findings that implicate the role of donor blood composition on the development of NAS after DCD liver transplantation. We hypothesized that donor

hematocrit, thrombocyte and leukocyte counts could influence the rates of NAS after transplantation. For example, hematocrit could affect the viscosity of the blood, platelets can play a role in endothelial activation, and similar effects can be attributed to leukocytes, which also can initiate local vasodilation, and all of these factors can influence graft flush-out, and thereby influence the severity of biliary injury. First, in a histological analysis of 40 bile duct biopsies of human donor livers, increased donor platelet counts and reduced leukocyte counts were associated with a high bile duct injury score (Odds Ratio 2.553, 95%CI 1.082-6.021, p=0.029 and Odds Ratio 0.734 95%CI 0.581-0.927, p=0.009, respectively). Secondly, during normothermic machine perfusion (NMP) of the abovementioned livers, grafts of donors with increased platelet count and reduced leukocyte count produced bile with lower biliary bicarbonate and pH levels, indicating more severe biliary injury. Lastly, in a nationwide retrospective database analysis, donor hematocrit and platelet count were identified as significant independent risk factors for the development of NAS after DCD liver transplantation (Hazard Ratio 1.047 95%CI 1.007-1.089, p=0.022 and Hazard Ratio 1.044 95%CI 1.005-1.083, p=0.025). Altogether, these data suggest that blood composition of the donor is associated with the development of biliary injury during DCD-LT.

In the study described in **Chapter 5**, we aimed to assess the safety of the use of DCD livers for patients that require a re-transplantation of the liver. In the Western world, DBD livers are the golden standard when it comes to graft selection for re-transplantation. However, over the last years, the three liver transplant centers in the Netherlands have performed several re-transplantations with a DCD liver. In this observational study, we compared the outcomes after DCD re-transplantation with a matched cohort of DBD re-transplantations. Interestingly, graft and patient survival were similar among both groups. Rates of NAS in the DCD cohort were high (38%), but the requirement for retransplantation for these NAS cases was low. This study suggests that for selected re-transplantation candidates, using a DCD graft from a young, healthy donor may lead to non-inferior outcomes. DCD livers should therefore not be routinely declined for re-transplantation candidates.

SUMMARY PART II:

The second part of this thesis contains studies aiming to expand the boundaries of transplantation of suboptimal donor livers.

In **Chapter 6**, a histological analysis was performed of bile duct biopsies of 10 DCD livers that underwent end-ischemic dual hypothermic machine perfusion (DHOPE) prior to graft implantation, compared with a historical cohort of 20 DCD livers without machine perfusion of which bile duct biopsies were taken. Upon baseline, prior to any intervention, no difference was observed between the DHOPE group and the control group. After graft reperfusion, livers that had underwent DHOPE displayed less stroma necrosis and less periluminal and deep peribiliary gland injury after reperfusion, compared to the control group of livers that were continuously preserved on ice. From this study, it was concluded that DHOPE attenuates the histological sequelae of ischemia-reperfusion injury of the biliary tree.

In **Chapter 7**, the results of a preclinical study on extended hypothermic dynamic preservation are presented. We investigated the safety of prolonged DHOPE by comparing liver graft function after 2, 6 and 24 hours of DHOPE. Following dynamic preservation of 2, 6 or 24 hours using DHOPE, reperfusion with autologous blood was performed and liver graft function was tested. Interestingly, no differences in lactate clearance, pH stabilization, bile production and bile composition were observed between the groups. All analyzed markers of endothelial distress and/or injury displayed no differences between the 2,6 and 24 hours of DHOPE groups. Additionally, as proof of concept two human livers declined for transplantation underwent hypothermic dynamic preservation using DHOPE for 20 hours prior to rewarming and reperfusion. These human liver grafts also showed excellent hepatobiliary viability during NMP. From this study, it was concluded that it seems safe to extend DHOPE for up to 24 hours. After confirmation in a transplantation-setting, this strategy may have substantial clinical impact because it may simplify the difficult logistics around transplants and can initiate day-time liver transplant surgery.

In **chapter 8**, we describe the results from a prospective clinical trial, the DHOPE-COR-NMP trial. In this study, all nationwide declined livers were offered for inclusion in the protocol. Mainly, the offered grafts were DCD livers with a donor age exceeding 60 years,

or DCD grafts with high percentage of steatosis. Livers first underwent one hour of DHOPE, to resuscitate the mitochondria and prevent subsequent ischemia-reperfusion injury. After 60 minutes of controlled oxygenated rewarming (COR) to 37°C, normothermic machine perfusion (NMP) was continued. Within the first 2.5 hours of NMP, livers had to meet the following criteria: perfusate lactate <1.8mmol/L, pH 7.35-7.45, bile production >10mL and biliary pH >7.45. Within the trial period, sixteen livers underwent DHOPE-COR-NMP. All livers showed adequate liver parenchymal function and cleared lactate and produced bile. However, only eleven of sixteen livers reached a bile pH >7.45 and were therefore suitable for safe transplantation. One-year graft and patient survival following these eleven transplants were 100%. With the DHOPE-COR-NMP protocol, 69% of livers that were previously discarded were successfully transplanted with a 100% graft survival at 12 months, resulting in an over 20% increase in the number of post-mortal liver transplantations in the University Medical Center Groningen.

The aim of the study in **chapter 9** was to find a method to simplify ischemia-free liver transplantation as performed by the transplant team in the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. In Guangzhou, a small interposition vein is placed end-to-side on the portal vein, to allow cannulation and continuous perfusion during procurement, preservation and implantation. We hypothesized that cannulation of the surgically reopened umbilical vein would allow continuous perfusion as well. To test this, we perfused five donor livers via the umbilical vein and monitored perfusion closely. The first three livers were perfused hypothermically, and adequate flows were observed with low pressures, similar to regular (D)HOPE. The next two livers were perfused normothermically. The first cleared lactate, normalized pH and produced high quality bile. This was also observed in the second liver, which was ‘split on the pump’. In this chapter, we have also described a method for splitting liver grafts ‘on the pump’ with the feasibility of subsequent ischemia-free implantation of the left lateral segment graft.

Discussion and future perspectives

Through the studies in this thesis we have attempted to elucidate the boundaries of transplantation of suboptimal donor livers. However, large parts of the grey area between transplantable and non-transplantable livers remain unexplored. Future studies can help recognize the pathophysiology behind complications that represent the actual boundaries. Once the mechanisms of these complications are fully understood, one can attempt to push the boundaries by applying novel therapies. In this part of the thesis, the results of the studies described will be discussed, and future perspectives for studies on suboptimal liver transplantation are considered.

In an attempt to introduce a chronological order to this thesis, **chapter 2** provided background and rationale for chapter 3-5. Biliary complications form the Achilles' heel of DCD liver transplantation. Over the last years, the pathophysiology of post-transplant cholangiopathy has been extensively studied.[1] Op den Dries et al showed in 2014 that biliary complications are largely predicted by the severity of histological injury observed upon arrival of the graft in the transplant center.[2] Two specific aspects of the biliary injury have a very strong predictive value for NAS: damage to the peribiliary glands (PBG's) and damage to the peribiliary vascular plexus (PVP) located in the bile duct wall. Interestingly, in the study by op den Dries, a large increase in biliary damage was observed directly after graft reperfusion in the recipient, demonstrating the impact of reperfusion injury following ischemia.[2] This offers the potential for a therapeutic intervention in the period between arrival of the organ and graft reperfusion, the so-called 'end-ischemic period', and this formed the rationale for initiating the clinical trial of which chapter 7 was a satellite study.[3] Possible end-ischemic interventions can attenuate reperfusion injury, and thereby reduce rates of NAS after transplantation.

However, in the absence of regenerative therapy, going beyond attenuating reperfusion injury is not yet possible. Future studies aiming at repairing the damage that is already present upon arrival of the graft, are eagerly awaited. As active repair is not yet feasible, some of the chapters in this thesis are focused on identifying mechanisms that influence the severity of biliary injury already before arrival in the transplant center. This includes **chapter 3,** where the donor hepatectomy time was identified as a crucial period in the development of biliary injury. If this period is kept short, biliary injury is minimized. However, over the last decades, training and regular feedback for organ procurement

teams has apparently been insufficient in the Netherlands. In the same period in the United Kingdom, regulations required a donor hepatectomy time to be below 45 minutes.[4] In the Netherlands, the median hepatectomy time over the last fifteen years has been 63 minutes, with cases exceeding two hours. This can partly explain the extremely high rates of NAS in the Netherlands that have been reported previously.[5-7] Future perspectives in the prevention of hepatectomy time induced biliary injury mainly comprises education of donor surgeons and introduction of normothermic regional perfusion (NRP). With NRP, impressive results have been observed in terms of post-transplant cholangiopathy rates and graft survival.[8,9] NRP is a method in which a perfusion device (e.g. extracorporeal membrane oxygenation device) is used to perfuse the abdominal (and potentially also the thoracic) cavity via large cannulas after circulatory arrest of the donor. This method quickly leads to restoration of intracellular adenosine triphosphate levels and hypothetically converts a DCD procedure into a more controlled procedure.[8,9] So far, no randomized controlled trials have been performed or initiated to our knowledge, but NRP appears to have the potential to reduce rates of biliary complications after DCD liver transplantation.

Another study on the mechanisms influencing biliary injury prior to arrival in the recipient transplant center is described in **chapter 4**, which provides a significant amount of fuel for future perspectives. There were two rationales for this study. First, it still remains uncertain whether the two aspects of biliary injury that predict post-transplant cholangiopathy, namely peribiliary gland and peribiliary vascular plexus injury, both simply occur simultaneously as a result of donor warm ischemia in the DCD donor, or that they occur sequential. So far, no studies have been performed involving bile duct biopsies taken upon organ procurement, but it would be highly interesting to see if both PBG and PVP injury are already present prior to static cold preservation (SCS), or whether they develop simultaneously during SCS. It can be hypothesized that if PVP injury would develop prior to PBG injury, perhaps PBG injury can be prevented when interventions against PVP injury are initiated.

Secondly, it is still uncertain whether the additional warm ischemia that DCD donors encounter in comparison to DBD donors is solely responsible for the increase in biliary injury following DCD liver transplantation. Other mechanisms that can lead to PVP injury are present in a DCD donor in the process of dying. Unfortunately, not much

research has been performed on how the vasculature responds to an approaching circulatory arrest and to the first minutes after cessation of blood flow. Severe peripheral vasoconstriction would for example lead to less effective flush-out and therefore potentially aggravate peripheral PVP injury. The circulatory arrest and subsequent blood stasis is also pro-thrombotic, and can cause flush-out problems by microthrombi formation.

However, interestingly, there has been quite a lot of research performed on the vascular response to hypoxia, which is largely dependent on endothelial cell function.[10-13] DCD donors experience a significant period of hypoxia prior to circulatory arrest. Endothelial cell function is dependent on many aspects, including age and atherosclerotic disease. It has been described that young healthy people respond to hypoxia with peripheral vasodilation, but that this response completely disappears as people age.[10] This can explain our observation that biliary injury upon arrival in the recipient center is more extreme in elderly donors. Over the last years, after identifying donor age as a risk factor for NAS, it has been hypothesized that after transplantation, older grafts have reduced regenerative capacity from the peribiliary glands. However, the observation that biliary injury is already more severe upon arrival in the recipient center prior to graft reperfusion, partially contradicts this hypothesis, and suggests involvement of other mechanisms earlier in the transplant process.

In chapter four, especially the donor platelet count appeared to be strongly correlated to the development of biliary injury. Platelets are known to induce endothelial dysfunction upon hypoxia and we therefore hypothesized in chapter 5 that increased platelet count leads to more vasoconstriction and less efficient arterial flush-out, and thereby influences biliary injury.[14] Minor et al observed early in this century that a pre-flush with streptokinase prior to regular ice-cold flushing improved graft viability.[15] As this is an easy intervention that can improve preservation of microvasculature, the effect of this donor pre-flush on biliary injury should be studied. However, hopefully, the need to study superior flushing techniques disappears when NRP becomes standard of care in DCD liver donors.

We hypothesized that the association between blood composition and post-transplant outcomes is particularly notable with respect to biliary complications. Liver grafts are flushed through the portal vein upon procurement, and therefore adequate cooling can

be achieved for the liver parenchyma even when the arterial flush did not adequately rinse the microcirculation. However, as the bile duct is largely dependent on the hepatic artery for blood supply, the importance of high-quality arterial flushing is essential in the prevention of biliary injury.

The study in **chapter 5** removed a small boundary that many centers held on to: that DCD livers by definition should not be accepted for patients requiring a re-transplantation. However, we observed no difference in graft survival between DCD livers used for re-transplantation and a matched cohort of DBD livers. We envision that when DHOPE will be used regularly, DCD livers can be easily accepted for stable patients listed for retransplantation. As in chapter 8 it is described that there is no difference in liver graft function after 2, 6 and 24 hours of DHOPE, the recipient hepatectomy in case of a retransplantation can be performed without time pressure when the organ is placed on the perfusion device.

The effects of DHOPE have been studied in several chapters in this thesis. In **chapter 6**, it was observed that DHOPE reduces reperfusion injury of the biliary tree. Moreover, in **appendix I** we described the first case of liver transplantation after hypothermic machine perfusion of a pediatric liver graft, with excellent outcome. Subsequently in **chapter 7**, DHOPE allowed safe preservation of liver grafts for up to 24 hours. This study provides rationale for initiating a day-time liver transplantation program. Every donor liver that is expected to arrive after, for example, 20:00, can be placed on the perfusion device until the next morning. Comparison with the intervention group of the DHOPE randomized controlled trial (short periods of DHOPE) can be made to adequately assess the safety of prolonged DHOPE. The benefit of day-time surgery is that an optimal team can be arranged and that the transplant team is well-rested.

With the DHOPE-COR-NMP protocol, in **chapter 8** all livers showed excellent hepatocellular viability. We are convinced that DHOPE is a safe technique and that centers should first introduce cold perfusion into the clinic, prior to initiating a NMP program. Starting a NMP program without proper experience in the field of machine perfusion is risky.[16] In contrast to other studies with NMP that attempt to assess graft function of discarded livers to allow transplantation, DHOPE-COR-NMP is so far the only protocol leading to 100% of livers meeting hepatocellular viability criteria.[17-19] Whether this is the result of the DHOPE preceding the NMP, should be studied in the future. Large

trials will have to demonstrate the direct effect of DHOPE on NAS and graft survival, and as two large trials in hypothermic machine perfusion have currently closed their inclusions, the results are eagerly awaited.[20,21] In **appendix II**, viability criteria that can be used to assess graft function during NMP are discussed.

The drawback of DCD liver transplantation remains the biliary injury. Even in the DHOPE-COR-NMP trial, 31% of the livers were considered 'non-viable' based on bile composition, which reflects bile duct viability. Including the one liver that developed NAS, that would with the current experience be declined for transplantation, actually 38% of the livers had too severe biliary injury prior to DHOPE-COR-NMP. Therefore, even with end-ischemic (D)HOPE for regular DCD livers, such as in the DHOPE-DCD randomized controlled trial, the percentage of NAS will not be zero, as there will be always be livers that already arrive with too severe biliary damage. Short donor hepatectomy and cold ischemia times can minimize the degree of biliary injury before implantation. In conclusion; DHOPE, and DHOPE-COR-NMP, have stretched the boundaries of transplantation of suboptimal donor livers. To push the boundaries even further, several interventions in the donor hospital should be studied, such as NRP.

Last to discuss is a very hopeful development in Guangzhou, China, where a transplant team succeeded in performing the first series of ischemia-free liver transplantation (IFLT) worldwide.[22] With this technique, biliary injury will hopefully no longer play a major role in liver transplantation. However, it will take some time before implementation in the Western world can begin, as a result of the complexity of the procedure and the logistics. In Guangzhou, all donors were transferred to the recipient hospital, which is not allowed in most Western countries. In a DCD setting, completely ischemia-free transplantation is not possible, as a result of the donor warm ischemia time. However, with the use of NRP further ischemia will be avoided and the risk of NAS after transplantation will be low. One way to simplify IFLT is described in **chapter 9**.

The studies described in this thesis aimed to allow safe transplantation of high-risk, suboptimal donor livers. However, still large amounts of donor livers are declined for transplantation. As long as a significant number of patients die on the waiting list for an organ, transplant physicians should study safe transplantation of these high-risk donor organs. As described in an editorial in the New England Journal of Medicine: "risk aversion has contributed to a shift in the research portfolios of many transplant

programs, away from discovery research and toward health services science and clinical outcomes studies".[23] The study aiming to allow safe transplantation of previously declined donor livers (chapter 8), demonstrates that a mutual risk taken by patient and physician can lead to a large step forward in the reduction of waiting list mortality. Many future studies are required to decrease waiting list mortality and reduce rates of complications after transplantation. To achieve this goal, physicians should not refrain from risks, but they should enter the bold path to medical advances together with the patient.

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